MARTIN & FERRARO, LLP
ATTORNEYS AT LAW
1557 Lake O'Pines Street, NE
Hartville, Ohio 44632

Telephone
(330) 877-0700

Facsimile
(330) 877-2030

FACSIMILE TRANSMITTAL

TO:

Name: Mail Stop APPEAL BRIEF-Patents
Group Art Unit 3773/Examiner Melanie Ruano Tyson

Firm: U.S. Patent & Trademark Office

Fax No.: 571-273-8300

Subject: U.S. Patent Application No. 10/098,683
Gary Karlin Michelson
Filed: March 15, 2002
SPINAL IMPLANT CONTAINING MULTIPLE BONE GROWTH PROMOTING MATERIALS (as amended)
Attorney Docket No. 101.0042-05000
Customer No. 22882
Confirmation No.: 7210

FROM:

Name: Thomas H. Martin, Esq.

Phone No.: 330-877-2277

No. of Pages (including this): 35

Date: August 1, 2008

Confirmation Copy to Follow: NO

Message:

CERTIFICATE OF TRANSMISSION UNDER 37 CFR 1.8

I hereby certify that the attached Transmittal Form (in duplicate; total amount of $510.00 to cover the Appeal Brief fee is to be charged to Deposit Account No. 50-3726) and Appeal Brief with Attachments A and B are being facsimile transmitted to the U.S. Patent and Trademark Office on August 1, 2008.

Sandra L. Blackmon

If there is a problem with this transmission please call the sender at the number above.

FORM PTO-1083

Attorney Docket No.: 101.0042-05000
Customer No. 22882

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Gary Karlin Michelson
Serial No: 10/098,683
Filed: March 15, 2002
For: SPINAL IMPLANT CONTAINING MULTIPLE BONE GROWTH PROMOTING MATERIALS (as amended)

Confirmation No.: 7210

Art Unit: 3773
Examiner: Melanie Ruano Tyson

Mail Stop APPEAL BRIEF-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Further to the Notice of Appeal filed May 29, 2008 and the Notice of Panel Decision from Pre-Appeal Brief Review dated July 1, 12008, transmitted herewith is an Appeal Brief in the above-identified application.

☐ No additional fee is required.

☐ Applicant hereby requests a ***-month extension of time to respond to the above Office Action.

☒ The total amount of $510.00 to cover the Appeal Brief fee is to be charged to Deposit Account No. 50-3726.

☒ The Commissioner is hereby authorized to charge any deficiencies of fees associated with this communication or credit any overpayment to Deposit Account No. 50-3726.
A copy of this sheet is enclosed.
☒ Any filing fees under 37 C.F.R. § 1.16 for the presentation of extra claims
☒ Any patent application processing fees under 37 C.F.R. § 1.17

Respectfully submitted,

MARTIN & FERRARO, LLP

Date: August 1, 2008

By: /Thomas H. Martin/
Thomas H. Martin
Registration No. 34,383

1557 Lake O'Pines Street, NE
Hartville, Ohio 44632
Telephone: 330-877-0700
Facsimile: 330-877-2030

FORM PTO-1083

Attorney Docket No.: 101.0042-05000
Customer No. 22882

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Gary Karlin Michelson
Serial No: 10/098,683
Filed: March 15, 2002
For: SPINAL IMPLANT CONTAINING MULTIPLE BONE GROWTH PROMOTING MATERIALS (as amended)

Confirmation No.: 7210

Art Unit: 3773
Examiner: Melanie Ruano Tyson

Mail Stop APPEAL BRIEF-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Further to the Notice of Appeal filed May 29, 2008 and the Notice of Panel Decision from Pre-Appeal Brief Review dated July 1, 12008, transmitted herewith is an Appeal Brief in the above-identified application.

☐ No additional fee is required.

☐ Applicant hereby requests a ***-month extension of time to respond to the above Office Action.

☒ The total amount of $510.00 to cover the Appeal Brief fee is to be charged to Deposit Account No. 50-3726.

☒ The Commissioner is hereby authorized to charge any deficiencies of fees associated with this communication or credit any overpayment to Deposit Account No. 50-3726.
A copy of this sheet is enclosed.
☒ Any filing fees under 37 C.F.R. § 1.16 for the presentation of extra claims
☒ Any patent application processing fees under 37 C.F.R. § 1.17

Respectfully submitted,

MARTIN & FERRARO, LLP

Date: August 1, 2008

By: /Thomas H. Martin/
Thomas H. Martin
Registration No. 34,383

1557 Lake O'Pines Street, NE
Hartville, Ohio 44632
Telephone: 330-877-0700
Facsimile: 330-877-2030

PATENT
Attorney Docket No. 101.0042-05000
Customer No. 22882

APPEAL TO THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of:	)	
Gary Karlin Michelson	)	Confirmation No.: 7210
Serial No.: 10/098,683	)	
Filed: March 15, 2002	)	
For: SPINAL IMPLANT CONTAINING MULTIPLE BONE GROWTH PROMOTING MATERIALS (as amended)	)	Group Art Unit: 3773 Examiner: Melanie Ruano Tyson

Mail Stop APPEAL BRIEF-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

APPEAL BRIEF

Real Party in Interest

The real party in interest is Warsaw Orthopedic, Inc. (hereinafter, the "Appellant").

Related Appeals and Interferences

There are no appeals or interferences pending which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

Status of Claims

Claims 1-53, 66, and 91 have been cancelled.

Claims 54-65, 67-90, and 92-108 are pending.

Claims 54-65, 67-90, and 92-108 have been rejected and are being appealed.

Status of Amendments

No amendments were filed subsequent to the Final Office Action dated March 14, 2008.

RECEIVED
CENTRAL FAX CENTER
AUG 0 1 2008

Summary of Claimed Subject Matter

Independent Claim 54.

The present invention in one preferred embodiment is directed to an apparatus comprising: an interbody spinal fusion implant (40) for surgical implantation within a disc space (D) between two adjacent vertebral bodies (V) in a segment of a human spine (Specification, first full paragraph on page 12, and Figs. 1 and 12), the implant (40) comprising upper and lower portions for contacting each of the adjacent vertebral bodies (V) when positioned therein (Figs. 1 and 12), each of the upper and lower portions having at least one opening adapted to communicate with one of the adjacent vertebral bodies (Fig. 12), the openings of the upper and lower portions being in communication with one another and adapted for permitting for the growth of bone from adjacent vertebral body to adjacent vertebral body through the implant (inherent nature of interbody spinal fusion implants, and Fig. 12), the implant (40) including a hollow interior for holding bone growth promoting material (inherent nature of interbody spinal fusion implants, and Fig. 12), the hollow interior being in communication with at least one opening in each of the upper and lower portions (Fig. 12), the implant (40) having an insertion end for entry into the spine and a trailing end, the trailing end having a rear wall between the upper and lower portions (Figs. 1, 4, and 12);

a liquid fusion promoting material in at least a portion of the hollow interior to promote bone growth from adjacent vertebral body to adjacent vertebral body through the implant (Specification, paragraph bridging pages 13 and 14, and Fig. 12 showing that a spinal fixation device (10) coated with bone fusion promoting materials is provided in at least a portion of the hollow interior); and

a solid fusion promoting material other than bone, the solid fusion promoting material being in at least a portion of the hollow interior to promote bone growth from adjacent vertebral body to adjacent vertebral body through the implant (Specification, paragraph bridging pages 13 and 14, and Fig. 12 showing that a spinal fixation device (10) coated with bone fusion promoting materials is provided in at least a portion of the hollow interior).

Independent Claim 79.

The present invention in another preferred embodiment is directed to an apparatus comprising: an interbody spinal fusion implant (40) for surgical implantation within a disc space (D) between two adjacent vertebral bodies (V) in a segment of a human spine (Specification, first full paragraph on page 12, and Figs. 1 and 12), the implant (40) comprising upper and lower portions for contacting each of the adjacent vertebral bodies (V) when positioned therein (Figs. 1 and 12), each of the upper and lower portions having at least one opening adapted to communicate with one of the adjacent vertebral bodies (Fig. 12), the openings of the upper and lower portions being in communication with one another and adapted for permitting for the growth of bone from adjacent vertebral body to adjacent vertebral body through the implant (inherent nature of interbody spinal fusion implants, and Fig. 12), the implant including a hollow interior for holding bone growth promoting material (inherent nature of interbody spinal fusion implants, and Fig. 12), the hollow interior being in communication with at least one opening in each of the upper and lower portions (Fig. 12), the implant having an insertion end for entry into the spine and a trailing end (Figs. 1, 4, and 12), the trailing end being adapted so as to be connectable to another interbody spinal implant (41) having a trailing end adapted to be connected to the interbody spinal fusion implant (40) (Specification, paragraph bridging pages 11 and 12, first paragraph on page 12, and Figs. 1 and 12);

a bioactive material in at least a portion of the hollow interior to promote bone growth from adjacent vertebral body to adjacent vertebral body through the implant (Specification, paragraph bridging pages 13 and 14, and Fig. 12 showing that a spinal fixation device (10) coated with bone fusion promoting materials is provided in at least a portion of the hollow interior); and

a bioresorbable material being in at least a portion of the hollow interior to promote bone growth from adjacent vertebral body to adjacent vertebral body through the implant (Specification, paragraph bridging pages 13 and 14, and Fig. 12 showing

that a spinal fixation device (10) coated with bone fusion promoting materials is provided in at least a portion of the hollow interior).

Dependent claims 67 and 104-106 depending from independent claim 54.

The present invention in other preferred embodiments is directed to the solid fusion promoting material including at least one of hydroxyapatite and hydroxyapatite tricalcium phosphate; the liquid fusion promoting material being bone morphogenetic protein; and the solid fusion promoting material including at least one of a bioactive material and a bioresorbable material (Specification, paragraph bridging pages 13 and 14).

Grounds of Rejection to be Reviewed on Appeal

I. Claims 54-65, 67-78, and 104-106 (including independent claim 54) stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

II. The Amendment filed June 18, 2007 stands objected to under 35 U.S.C. § 132(a) for introducing new matter to the Abstract.

III. Claims 54-65, 67-90, and 92-108 (including independent claims 54 and 79) stand rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,026,373 to Ray et al. ("Ray").

Argument

Appellant submits the following arguments for consideration by the Board of Patent Appeals and Interferences:

I. Rejection of claims 54-65, 67-78, and 104-106 (including independent claim 54) under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

In rejecting claims 54-65, 67-78, and 104-106 under 35 U.S.C. § 112, first paragraph, the Examiner indicates that "Appellant failed to disclose a liquid fusion promoting material and a solid fusion promoting material at the time the application was filed (see claims 54, 67, and 104-106)." (Final Office Action dated March 14, 2008, paragraph bridging pages 2 and 3.) Furthermore, in support of the rejection under 35 U.S.C. § 112, first paragraph, the Examiner indicated that , "the terms 'liquid' and 'solid'

cover other materials in addition to those disclosed by the Appellant," and "although the materials disclosed by the Appellant may inherently contain these properties, the Appellant did not disclose all liquid and solid fusion promoting materials." (Final Office Action of March 14, 2008, second full paragraph on page 5.) According to the Examiner, "Appellant simply disclosed bone fusion promoting material, such as hydroxyapatite, tricalcium phosphate, and bone morphogenetic protein," and "[t]herefore, claims 54-65, 67-78, and 104-106 contain new matter." (Final Office Action dated March 14, 2008, paragraph bridging pages 2 and 3.)

In response, Appellant submits that the solid states of hydroxyapatite and hydroxyapatite tricalcium phosphate, and the liquid state of bone morphogenetic protein (BMP) are inherent properties of those materials when used to promote bone fusion. Furthermore, Appellant is not required to disclose all liquid and solid fusion promoting materials to support the recitations of claims 54, 67, and 104-106. Instead, Appellant submits that one of ordinary skill in the art at the time application was filed would have understood that Appellant disclosed solid and liquid fusion promoting materials, and would conclude that Appellant was in possession of the invention as claimed. As discussed below, because no new matter has been introduced into the claims, the Examiner has failed to make a prima facie case for the rejection of claims 54-65, 67-78, and 104-106 under 35 U.S.C. § 112, first paragraph.

According to MPEP § 2163.III.A, a prima facie case under 35 U.S.C. § 112, first paragraph, requires that the Examiner provide "reasons why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure of the application as filed." Appellant notes that MPEP § 2163 II.A.3(b) indicates that "each claim limitation must be expressly, implicitly, or inherently supported in the originally filed disclosure" to comply with the written description requirement of 35 U.S.C. § 112, first paragraph. According to MPEP § 2163 II.A.3(b), citing Hyatt v. Boone, 146 F.3d 1348 (Fed. Cir. 1998), "[w]hen an explicit limitation in a claim 'is not present in the written description whose benefit is sought it must be shown that a person of ordinary skill would have

understood, at the time the patent application was filed, that the description requires that limitation.'" Thereafter, MPEP § 2163 II.A.3(b) cites the following precedential example where claims can and cannot derive inherent support from the specification:

> To establish inherency, the extrinsic evidence "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient. (MPEP § 2163 II.A.3(b), citing *In re Robertson*, 169 F.3d 743 (Fed. Cir. 1999).)

As discussed below, Appellant submits that independent claim 54, and dependent claims 67 and 104-106 are supported by the inherent properties of hydroxyapatite, tricalcium phosphate, and bone morphogenetic protein.

Hydroxyapatite, hydroxyapatite tricalcium phosphate, and BMP are disclosed in Appellant's Specification as bone fusion promoting materials. (See Specification, paragraph bridging pages 13 and 14.) Both hydroxyapatite and hydroxyapatite tricalcium phosphate are generally used in solid form as fusion promoting materials, and BMP is generally used in liquid form as a fusion promoting material. In fact, in the case of the implant coatings referenced in Appellant's Specification, Appellant submits that, as used to promote bone fusion, the solid states of hydroxyapatite and hydroxyapatite tricalcium phosphate, and the liquid state of BMP are inherent, not probable or possible, properties of those materials. Table 1 lists scholarly articles confirming the above-discussed inherent properties of hydroxyapatite, hydroxyapatite tricalcium phosphate, and BMP.

MATERIAL	STATE DURING USE	REFERENCE
Hydroxyapatite	Solid	See, e.g., Comparison of Hydroxyapatite and Hydroxyapatite Tricalcium-Phosphate Coatings, The Journal of Arthroplasty, Volume 17, Issue 7, Pages 902-909, T. Jinno. Included as Attachment A.
Hydroxyapatite Tricalcium Phosphate	Solid	See, e.g., Comparison of Hydroxyapatite and Hydroxyapatite Tricalcium-Phosphate Coatings, The Journal of Arthroplasty, Volume 17, Issue 7, Pages 902-909, T. Jinno. Included as Attachment A.
Bone Morphogenetic Protein	Liquid	See, e.g. Bone morphogenetic proteins: basic concepts, Neurosurg Focus, Volume 13 (6), Pages 1-6, S.S. Rengachary SS. Included as Attachment B.

As discussed in the first of the above-referenced scholarly articles (Attachment A), hydroxyapatite and hydroxyapatite tricalcium phosphate are used in solid form when used to promote bone fusion. Furthermore, as discussed in the second of the above-referenced scholarly articles (Attachment B), BMP is used in liquid form when used to promote fusion. The second above-referenced scholarly article (Attachment B) indicates that BMP is generally used with a carrier such as hydroxyapatite and hydroxyapatite tricalcium phosphate.

The field of spinal surgery is an art of relatively high skill and knowledge. One of ordinary skill in the art of spinal surgery would recognize the inherent properties of hydroxyapatite, hydroxyapatite tricalcium phosphate, and BMP when used to promote bone fusion. Accordingly, one of ordinary skill in the art of spinal surgery would conclude, given their inherent properties, that the disclosure of hydroxyapatite, hydroxyapatite tricalcium phosphate, and BMP support the invention as claimed. That is, given the inherent properties of hydroxyapatite, hydroxyapatite tricalcium phosphate, and BMP, Appellant submits that a person of ordinary skill in the art of spinal surgery would conclude that Appellant was in possession of the invention as claimed. Therefore, no new matter has been introduced into the claims. Accordingly, the Examiner has not made a prima facie case for the rejection of claims 54-65, 67-78, and 104-108 under 35 U.S.C. § 112, first paragraph, and Appellant submits the Examiner's rejection thereunder cannot be maintained.

II. Objection to the Amendment filed June 18, 2007 under 35 U.S.C. § 132(a).

The Examiner objected under 35 U.S.C. § 132(a) to amendments to the Abstract included in Appellant's Office Action response of June 18, 2007 for introducing new matter into the disclosure. In doing so, the Examiner indicates that "[t]he added material which is not supported by the original disclosure is as follows: In one embodiment, the bone growth promoting material includes a liquid fusion promoting material and a solid fusion promoting material other than bone provided in at least one portion of the hollow interior to promote growth from adjacent vertebral body to adjacent

vertebral body through the implant." (Final Office Action dated March 14, 2008, second full paragraph on page 2.) However, given that claims 54-65, 67-78, and 104-106 are patentable over the Examiner's rejection under 35 U.S.C. § 112, first paragraph, Appellant submits that the Abstract is adequately supported by the original disclosure. Hence, no new matter has been introduced in the Abstract, and the Examiner's objection to the June 18, 2007 Amendment under 35 U.S.C. § 132 has been overcome.

III. Rejection of claims 54-65, 67-90, and 92-108 (including independent claims 54 and 79) under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,026,373 to Ray.

The Examiner indicates that "it would have been obvious to one having ordinary skill in the art at the time the application was filed to employ the bone growth promoting materials as claimed in Ray's implant in order to promote new bone growth, since it has been held to be within the general skill of a worker in the art to select a known material on the basis of its suitability for the intended use as a matter of design choice." (Final Office Action dated March 14, 2008, paragraph bridging pages 3 and 4.) As discussed below, Appellant submits that the Examiner's rejection of independent claims 54 and 79 under 35 U.S.C. § 103(a) cannot be maintained.

In KSR International Co. v. Teleflex Inc. et al., the Supreme Court reaffirmed the framework for governing obviousness under 35 U.S.C. § 103(a) is set forth in Graham et al. v. John Deere Co. of Kansas City et al., 383 U.S. 1, 148 U.S.P.Q. 459 (1966). (See KSR v. Teleflex, 127 S.Ct. 1727 (2007)). Under Graham v. John Deere, the question of obviousness is resolved on the basis of factual determinations including (1) the scope and content of the prior art, (2) the differences between the claimed invention and the prior art, (3) the level of ordinary skill in the pertinent art, and (4) where in evidence, so-called secondary considerations. (Graham v. John Deere, at 17-18, 148 U.S.P.Q. at 467). However, even under Graham v. John Deere, a reference that does not teach or suggest every element of the claimed invention supports a finding of nonobviousness. Moreover, a finding of nonobviousness is also supported where the level of ordinary skill in the art would not afford modification of the reference to result in the claimed invention.

Independent claim 54 recites an apparatus comprising an interbody spinal fusion implant, and a liquid fusion promoting material and a solid fusion promoting material provided in the hollow interior of the implant, and independent claim 79 recites an apparatus comprising an interbody spinal fusion implant, and a bioactive material and a bioresorbable material provided in the hollow interior of the implant. Unlike Independent claims 54 and 79, however, Ray only discloses use of bone chips or other bone-inducing substance (the only one of which disclosed being cancellous bone). (See Ray, Abstract and column 10, lines 9-12.) According to Ray, fusion cages (10) and (50), for example, can be packed with the bone chips or cancellous bone. (See Ray, Abstract and column 10, lines 9-12.) Although the bone chips or cancellous bone are solid and bioresorbable materials, the bone chips or cancellous bone are not liquid or bioactive materials. Accordingly, Ray does not teach or suggest an interbody spinal fusion implant combined with liquid and solid fusion promoting materials as recited in independent claim 54, or an interbody spinal fusion implant combined with bioactive and bioresorbable materials as recited in independent claim 79. As such, Ray does not teach or suggest every element of Appellant's invention as recited in independent claims 54 and 79.

Furthermore, besides making the assertion that "It would have been obvious to one having ordinary skill in the art at the time the application was filed to employ the bone growth promoting materials as claimed in Ray's implant to promote new bone growth (emphasis added)," the Examiner has not pointed to any teaching or suggestion in the prior art affording such an assertion. That is, the Examiner has not indicated any support for the modification of Ray. Moreover, the Examiner overestimates the level of ordinary skill in the art at the time the application was filed. The present application has a priority date of March 28, 1994. The references, U.S. Patent Nos. 5,344,654 (Rueger) and 5,344,457 (Pillar), cited by the Examiner to show the level of ordinary skill in the art are not related to spinal fusion, and do not disclose providing a liquid fusion promoting material and a solid fusion promoting material in the hollow interior of an implant or providing a bioactive material and a bioresorbable material in the hollow

interior of an implant. Instead, the exteriors of the implants of Rueger and Pillar include bone growth promoting materials. Therefore, while Appellant submits that one of ordinary skill in the art of spinal surgery would recognize, as discussed above, the solid states of hydroxyapatite and hydroxyapatite tricalcium phosphate, and the liquid state of BMP, it would not have been obvious to one of ordinary skill in the art to modify Ray to use the liquid and solid fusion promoting materials (of independent claim 54) and the bioactive and bioresorbable materials (of independent claim 79) at the time the application was filed.

Additionally, the apparatus of independent claim 79 further recites that the interbody spinal fusion implant includes an insertion end for entry into the spine and a trailing end, where the trailing end is adapted so as to be connectable to another interbody spinal implant. Although the fusion cages (10) and (50) can include end caps (16) and (57), respectively, neither these fusions cages nor end caps are adapted so as to be connectable to another fusion cage. As such, Ray again does not teach or suggest every element of the Appellant's invention as recited in independent claim 79.

Given that Ray does not teach or suggest every element of Appellant's invention as recited in independent claims 54 and 78, and that it would not have been obvious to one of ordinary skill in the art to modify Ray to use the materials as recited in independent claims 54 and 79 at the time the application was filed, Appellant submits that the Examiner's rejection under 35 U.S.C. § 103(a) of independent claims 54 and 79 cannot be maintained. Accordingly, Appellant submits that independent claims 54 and 79 are not obvious in view of the Examiner's rejection under 35 U.S.C. § 103(a) based on Ray.

IV. Conclusion

Appellant submits that independent claims 54 and 79 are patentable and that dependent claims 55-65, 67-78, 80-90, and 92-108, dependent from one of independent claims 54 and 79, or claims dependent therefrom, are patentable at least due to their dependency from an allowable independent claim. Therefore, Appellant respectfully requests the Board to reverse the Examiner's rejections and objection, and allow claims 54-65, 67-90, and 92-108.

To the extent any extension of time under 37 C.F.R. § 1.136 is required to obtain entry of this Appeal Brief, such extension is hereby respectfully requested. If there are any fees due under 37 C.F.R. §§ 1.16 or 1.17 which are not enclosed herewith, including any fees required for an extension of time under 37 C.F.R. § 1.136, please charge such fees to our Deposit Account No. 50-3726.

Respectfully submitted,

MARTIN & FERRARO, LLP

Dated: August 1, 2008

By: /Thomas H. Martin/
Thomas H. Martin
Registration No. 34,383

1557 Lake O'Pines Street, NE
Hartville, Ohio 44632
Telephone: (330) 877-0700
Facsimile: (330) 877-2030

PATENT
Attorney Docket No. 101.0042-05000
Customer No. 22882

CLAIMS APPENDIX

Claims 1-53 (cancelled).

54. An apparatus comprising:

an interbody spinal fusion implant for surgical implantation within a disc space between two adjacent vertebral bodies in a segment of a human spine, said implant comprising upper and lower portions for contacting each of the adjacent vertebral bodies when positioned therein, each of said upper and lower portions having at least one opening adapted to communicate with one of the adjacent vertebral bodies, said openings of said upper and lower portions being in communication with one another and adapted for permitting for the growth of bone from adjacent vertebral body to adjacent vertebral body through said implant, said implant including a hollow interior for holding bone growth promoting material, said hollow interior being in communication with at least one opening in each of said upper and lower portions, said implant having an insertion end for entry into the spine and a trailing end, said trailing end having a rear wall between said upper and lower portions;

a liquid fusion promoting material in at least a portion of said hollow interior to promote bone growth from adjacent vertebral body to adjacent vertebral body through said implant; and

a solid fusion promoting material other than bone, said solid fusion promoting material being in at least a portion of said hollow interior to promote bone growth from adjacent vertebral body to adjacent vertebral body through said implant.

55. The apparatus of claim 54, wherein at least a portion of said upper and lower portions are arcuate along at least a portion of their length.

56. The apparatus of claim 54, wherein said upper and lower portions further comprise a protrusion for engaging the adjacent vertebral bodies.

57. The apparatus of claim 56, wherein said protrusion is a thread.

58. The apparatus of claim 54, wherein said insertion end is open for loading bone growth promoting material into said hollow interior.

59. The apparatus of claim 58, further comprising an end cap for closing said open end.

60. The apparatus of claim 54, wherein said hollow interior is a chamber and the bone growth promoting material includes a bone graft.

61. The apparatus of claim 54, wherein said implant is configured for implantation across the disc space in the thoracolumbar region of the human spine.

62. The apparatus of claim 54, wherein said spinal implant includes an artificial material other than bone.

63. The apparatus of claim 54, wherein said implant is made of an artificial material that is stronger than bone.

64. The apparatus of claim 54, wherein said implant is made of an artificial material that is harder than bone.

65. The apparatus of claim 54, wherein said implant comprises harvested bone.

Claim 66 (cancelled).

67. The apparatus of claim 54, wherein said solid fusion promoting material includes at least one of hydroxyapatite and hydroxyapatite tricalcium phosphate.

68. The apparatus of claim 54, wherein said implant is treated with a bone growth promoting substance.

69. The apparatus of claim 54, wherein said implant is a source of osteogenesis.

70. The apparatus of claim 54, wherein said implant is at least in part bioabsorbable.

71. The apparatus of claim 54, wherein said implant comprises at least one of a metal, a plastic material, and a ceramic material.

72. The apparatus of claim 54, wherein said upper and lower portions of said implant have a non-threaded exterior surface.

73. The apparatus of claim 54, wherein said implant has a length, said upper and lower portions having a non-arcuate portion along at least a portion of the length of said implant.

74. The apparatus of claim 54, wherein said implant is formed of a porous material.

75. The apparatus of claim 54, wherein said implant is formed of a material that intrinsically participates in the growth of bone from adjacent vertebral body to adjacent vertebral body through said implant.

76. The apparatus of claim 54, wherein said at least one opening is adapted to retain fusion-promoting materials

77. The apparatus of claim 54, wherein at least a portion of said implant is treated to promote bone ingrowth between said implant and said adjacent vertebral bodies.

78. The apparatus of claim 54, wherein said implant is in combination with harvested bone.

79. An apparatus comprising:

an interbody spinal fusion implant for surgical implantation within a disc space between two adjacent vertebral bodies in a segment of a human spine, said implant comprising upper and lower portions for contacting each of the adjacent vertebral bodies when positioned therein, each of said upper and lower portions having at least one opening adapted to communicate with one of the adjacent vertebral bodies, said openings of said upper and lower portions being in communication with one another and adapted for permitting for the growth of bone from adjacent vertebral body to adjacent vertebral body through said implant, said implant including a hollow interior for holding bone growth promoting material, said hollow interior being in communication with at least one opening in each of said upper and lower portions, said implant having an insertion end for entry into the spine and a trailing end, said trailing end being adapted so as to be connectable to another interbody spinal implant having a trailing end adapted to be connected to said interbody spinal fusion implant;

a bioactive material in at least a portion of said hollow interior to promote bone growth from adjacent vertebral body to adjacent vertebral body through said implant; and

a bioresorbable material being in at least a portion of said hollow interior to promote bone growth from adjacent vertebral body to adjacent vertebral body through said implant.

80. The apparatus of claim 79, wherein at least a portion of said upper and lower portions are arcuate along at least a portion of their length.

81. The apparatus of claim 79, wherein said upper and lower portions further comprise a protrusion for engaging the adjacent vertebral bodies.

82. The apparatus of claim 81, wherein said protrusion is a thread.

83. The apparatus of claim 79, wherein said insertion end is open for loading bone growth promoting material into said hollow interior.

84. The apparatus of claim 83, further comprising an end cap for closing said open end.

85. The apparatus of claim 79, wherein said hollow interior is a chamber and the bone growth promoting material includes a bone graft.

86. The apparatus of claim 79, wherein said implant is configured for implantation across the disc space in the thoracolumbar region of the human spine.

87. The apparatus of claim 79, wherein said spinal implant includes an artificial material other than bone.

88. The apparatus of claim 79, wherein said implant is made of an artificial material that is stronger than bone.

89. The apparatus of claim 79, wherein said implant is made of an artificial material that is harder than bone.

90. The apparatus of claim 79, wherein said implant comprises harvested bone.

Claim 91 (cancelled).

92. The apparatus of claim 79, wherein said bioactive material includes at least one of hydroxyapatite and bone morphogenetic protein.

93. The apparatus of claim 79, wherein said implant is treated with a bone growth promoting substance.

94. The apparatus of claim 79, wherein said implant is a source of osteogenesis.

95. The apparatus of claim 79, wherein said implant is at least in part bioabsorbable.
96. The apparatus of claim 79, wherein said implant comprises at least one of a metal, a plastic material, and a ceramic material.
97. The apparatus of claim 79, wherein said upper and lower portions of said implant have a non-threaded exterior surface.
98. The apparatus of claim 79, wherein said implant has a length, said upper and lower portions having a non-arcuate portion along at least a portion of the length of said implant.
99. The apparatus of claim 79, wherein said implant is formed of a porous material.
100. The apparatus of claim 79, wherein said implant is formed of a material that intrinsically participates in the growth of bone from adjacent vertebral body to adjacent vertebral body through said implant.
101. The apparatus of claim 79, wherein said at least one opening is adapted to retain fusion-promoting materials
102. The apparatus of claim 79, wherein at least a portion of said implant is treated to promote bone ingrowth between said implant and said adjacent vertebral bodies.
103. The apparatus of claim 79, wherein said implant is in combination with harvested bone.
104. The apparatus of claim 67, wherein said liquid fusion promoting material is bone morphogenetic protein.
105. The apparatus of claim 54, wherein said liquid fusion promoting material is bone morphogenetic protein.
106. The apparatus of claim 54, wherein said solid fusion promoting material includes at least one of a bioactive material and a bioresorbable material.
107. The apparatus of claim 92, wherein, when said bioresorbable material is tricalcium phosphate, said bioactive material is hydroxyapatite.
108. The apparatus of claim 79, wherein, when said bioresorbable material is tricalcium phosphate, said bioactive material is hydroxyapatite.

PATENT
Attorney Docket No. 101.0042-05000
Customer No. 22882

EVIDENCE APPENDIX

Attached hereto are Exhibits A and B, which appear in the record as part of the December 19, 2007 Reply. Current Exhibits A and B correspond to Exhibits A and B, respectively, of the December 19, 2007 Reply.

PATENT
Attorney Docket No. 101.0042-05000
Customer No. 22882

RELATED PROCEEDINGS APPENDIX

NONE.

Attachment A

The Journal of Arthroplasty Vol. 17 No. 7 2002

Comparison of Hydroxyapatite and Hydroxyapatite Tricalcium-Phosphate Coatings

Tetsuya Jinno, MD, PhD,* Dwight T. Davy, PhD,† and Victor M. Goldberg, MD*

Abstract: This study compared the effects of hydroxyapatite (HA) coating and biphasic HA/tricalcium-phosphate (HA/TCP) coating on the osseointegration of grit-blasted titanium-alloy implants. Each coated implant was compared with uncoated grit-blasted implants as well. The implants were press-fit into the medullary canal of rabbit femora, and their osseointegration was evaluated 3 to 24 weeks after surgery. The coated implants had significantly ($P<.05$) greater new bone ongrowth than the uncoated implants (HA, 56.1 ± 3.1%; HA/TCP, 53.8 ± 2.6%; uncoated, 32.2 ± 1.4% of the implant perimeter, 12 weeks). Unmineralized tissue (cartilage and osteoid) was seen on the uncoated implants but never on the coated implants. The coated implants had significantly ($P<.05$) greater interfacial shear strength than the uncoated implants (HA, 4.1 ± 0.4 MPa; HA/TCP, 4.8 ± 0.5 MPa; uncoated, 2.6 ± 0.2 MPa, 12 weeks). There was no difference between HA and HA/TCP coating in regard to new bone growth or interfacial shear strength. These data show a comparable enhancement effect of HA and HA/TCP coatings on the osseointegration of titanium-alloy implants. **Key words:** hydroxyapatite (HA) coating, hydroxyapatite/tricalcium-phosphate (HA/TCP), implant fixation, osseointegration, bone ongrowth.

Hydroxyapatite (HA) coating and biphasic calcium-phosphate (HA and tricalcium-phosphate [HA/TCP]) coating have been used clinically to enhance implant osseointegration. Intermediate clinical results of total hip arthroplasty using HA-coated implants are promising [1]. It is generally known that TCP is more soluble than HA at physiologic pH and more susceptible to bioresorption [2]. Partial dissolution of the calcium-phosphate ceramic macrocrystals followed by an increase in the calcium and phosphate ion concentrations in the local environment is thought to be important for the excellent osteoconductivity and tight *chemical bonding* of the bioactive ceramics with bone [3]. Although the greater, unpredictable solubility of the TCP coating may cause earlier failure of an HA/TCP-coated implant at the bone–implant interface [4], gradual resorption of this coating and replacement with new bone might be desirable to prevent the late complications of calcium-phosphate coatings [5,6]. One study using a canine unloaded implant model showed stronger fixation and higher bone ongrowth when HA-coated titanium-alloy implants were compared with TCP-coated implants [2]. By contrast, solid HA ceramics containing TCP induced better osseointegration than pure HA ceramics when implanted into sheep femora [7]. To our knowledge, no study has directly compared the *in vivo* effects of HA and HA/TCP used as coatings for implants.

The purpose of this study was to compare directly in a well-documented rabbit intramedullary

*From the *Department of Orthopaedics, Case Western Reserve University School of Medicine; and †Department of Mechanical and Aerospace Engineering, Case Western Reserve University, Cleveland, Ohio.*
Submitted January 18, 2001; accepted April 10, 2002.
Funds were received in partial or total support of the research material described in this article from Zimmer.
Reprint requests: Tetsuya Jinno, MD, PhD, Department of Orthopaedic Surgery, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. E-mail: jinno.orth@tmd.ac.jp

0883-5403/02/1707-0023$35.00/0
doi:10.1054/arth.2002.34821

Table 1. Characterization of the Surface

	Uncoated	HA-Coated	HA/TCP-Coated
Mean surface roughness (μm)	4.04 ± 0.51	4.09 ± 0.29	9.75 ± 1.13
Mean depth of roughness (μm)	24.48 ± 3.21	24.53 ± 2.12	50.59 ± 6.14
Maximum depth of roughness (μm)	33.53 ± 6.53	30.17 ± 4.80	67.46 ± 13.69

NOTE. Values are given as mean ± SD.

model [8,9] the effects of the HA and the HA/TCP coatings on osseointegration of titanium-alloy implants. The efficacy of these coatings for osseointegration of titanium-alloy implants also was evaluated by comparison with uncoated grit-blasted titanium-alloy implants, using the data from a previous study [9].

Materials and Methods

Implants

Solid, cylindrical rods (5 mm in diameter, 25 mm in length) of titanium-alloy (Ti-6Al-4V, ASTM F136) were used. Each rod had 1 threaded distal end (4 mm × 4 mm) and a capped portion on the proximal end (4 mm × 2 mm). The surface of the rods was grit-blasted with 24-grit aluminum-oxide particles [9]. After cleaning ultrasonically in purified water and passivating according to ASTM F86 standards, the rods were plasma-spray coated with HA or HA/TCP (ASTM F1185) (Zimmer, Warsaw, IN). The ratio of HA and TCP in the HA/TCP coating was 65%/35%, and the major structure of TCP was the β-TCP form. The crystalline phase material of HA was 90% at the least. The thickness of the coatings, determined by the manufacturer, ranged from 30 to 80 μm for HA and 50 to 125 μm for HA/TCP. These thicknesses were considered to be within an appropriate range in terms of bioresorption of the coating and tensile and fatigue strength of the coating [1,10,11]. The surface roughness before and after coating was measured by noncontact surface profilometry using a Perthometer S8P (Feinprüf Perthen GMBH, Göttingen, Germany) (Table 1). All implants were sterilized by gamma irradiation.

Animals and Surgical Procedure

Thirty-six skeletally mature male New Zealand White rabbits, weighing 3.2 to 4.0 kg, had bilateral insertion of an implant into the medullary canal of the distal femur. HA-coated and HA/TCP-coated rods were randomized to one or the other side, for a total of 72 implants. Two groups of 6 rabbits were sacrificed at 12 and 24 weeks postoperatively, and shear strength and failure pattern of the implant–bone interface were evaluated. Four groups of 6 rabbits were sacrificed at 3, 6, 12, and 24 weeks postoperatively, and the implants were evaluated histologically. This study was approved by the Institutional Animal Care and Use Committee.

The rabbits were anesthetized by intramuscular injection of ketamine (40 mg/kg), xylazine (5 mg/kg), and acepromazine (0.75 mg/kg). Cefazolin (80 mg) was administered intramuscularly immediately before the operation and on the first day postoperatively. Under sterile technique, a medial parapatellar arthrotomy was made, and a cylindrical hole was drilled into the intercondylar notch of the distal femur. The canal was reamed manually, and the implant was press-fit. The distal portion of the implant was within the metaphysis, and the proximal portion was within the diaphysis, in contact with the endosteal surface of the anterior and posterior cortex. These implants were not subjected to axial loading. Postoperatively, there were no restrictions on walking.

There were 3 intraoperative femoral fractures, and additional rabbits were used to complete the numbers of animals in each group. The rabbits were killed with an overdose of intravenous pentobarbital. The femora were retrieved, and radiographs were obtained using the Faxitron (Faxitron X-ray Corporation, Buffalo Grove, IL).

Histologic Examination

The 24 rabbits in the histologic study group were injected with fluorochromes every 10 days using the following schedule: doxycycline (5 mg/kg) on the 2nd, 12th, 82nd, 92nd, 142nd, and 152nd day; 2,4-bis-(N,N'-di-carboxymethyl-aminomethyl)-fluorescein (DCAF) (20 mg/kg) on the 22nd, 32nd, 122nd, and 132nd day; xylenol orange (90 mg/kg) on the 42nd, 52nd, 102nd, and 112th day; and alizarin complexone (30 mg/kg) on the 62nd, 72nd, and 162nd day. The retrieved distal femora were fixed in ethanol and embedded in methyl methacrylate. Undecalcified serial cross-sections were cut with a water-cooled diamond saw as previously

described [8]. The cross-sections were mounted on Plexiglas slides, then ground and polished on a precision grinder (Isomet; Buehler Corporation, Lake Bluff, IL) to a thickness of 50 to 80 μm. Two sections from each of diaphysis, proximal metaphysis, and distal metaphysis (6 sections for 1 femur) were surface-stained with toluidine blue for qualitative and quantitative analysis by light microscopy. Some sections were surface-stained with von Kossa stain. Other sections were left unstained for examination under epifluorescent light and scanning electron microscope in the backscatter mode (JSM-840A; JEOL, Tokyo, Japan) connected to x-ray energy dispersive spectroscopy (Series II X-ray Analyzer; Noran Instruments, Middleton, WI).

Operator-assisted histomorphometry (BioQuant TCW95 2.00MT; R & M Biometrics, Nashville, TN) was used for the blinded quantification of the tissue response around the implants. The new bone area within the circumferential zone of 0.5 mm around the implant was measured, and the percentage of the new bone area was calculated for each level. The new bone area was identified by its staining characteristics and morphology. The length of the implant surface in contact with the new bone also was measured for each level. The implant circumference was measured to allow calculation of the percentage of the new bone contact with the implant surface.

Mechanical Tests

The shear strength of the bone–implant interface was determined with a pull-out test. Testing was done within 24 hours after death. The specimen was mounted in a block of methyl methacrylate, leaving the distal threads exposed to connect to the servohydraulic testing machine (model 1320; Instron, Canton, MA). A tensile force was applied parallel to the long axis of the implant at a constant rate of displacement of 2 mm/min, and force-versus-displacement curves were recorded. An X-Y slide-table allowed the specimen to self-align to ensure that only a tensile load was applied to the implant. The peak load before failure at the bone–implant interface was divided by the exterior surface area of the implant to provide a measurement of the interface strength. To analyze the failure pattern, post-test specimens were processed by the same method as described for the slide preparation for histologic examination.

Comparison With Uncoated Titanium-Alloy Implants

Uncoated, grit-blasted titanium-alloy implants identical in all details to those reported herein were placed in the same position in the intramedullary canal of the distal femur of rabbits weighing 3.5 to 4.1 kg. The response to these implants was studied radiographically, histologically, histomorphometrically (3, 6, 12 weeks after surgery; n = 6 at each time period), and mechanically (12 weeks after surgery, n = 6) by exactly the same techniques as described here and was compared with the results of the HA-coated and the HA/TCP-coated implants. Some of the data of the uncoated implants were published previously [9].

Statistical Analysis

All of the data of coated and uncoated implants were entered into a single spreadsheet, and the means and standard errors of the means were calculated. The data were analyzed statistically with analysis of variance (independent variables were type of coating, time after implantation, and level of cross-section for histomorphometric analysis) using SPSS 7.5 for Windows (SPSS Inc, Chicago, IL). *Post hoc* assessment of significant ($P<.05$) differences was done with the Student-Newman-Keuls tests for comparison among the HA-coated, HA/TCP-coated, and uncoated specimens and paired *t*-tests with Bonferroni's correction for comparison between the HA-coated and HA/TCP-coated specimens.

Results

Radiographic Results

Microradiographs showed all implants to be well integrated, with no evidence of fractures, bony resorption, or radiolucent lines. By 6 weeks, bony bridges were noted to extend from the endosteum onto the implants, and these bony bridges became more apparent with time. No distinct radiographic differences were noted among the implants.

Qualitative Histologic Analysis

Active bone formation around and directly on the implant were noted with each type of implant, particularly at the posterior and anterior quadrants close to the endosteum. The new bone formation producing the bony bridges between the implants and cortex was more apparent on the coated implants than on the uncoated implants. The new bone, which was woven at 3 weeks, became thicker and was remodeled into lamellar bone with time. No inflammatory reaction was seen around any implant. At the bone–implant interface, however, there was an apparent difference of the pattern of

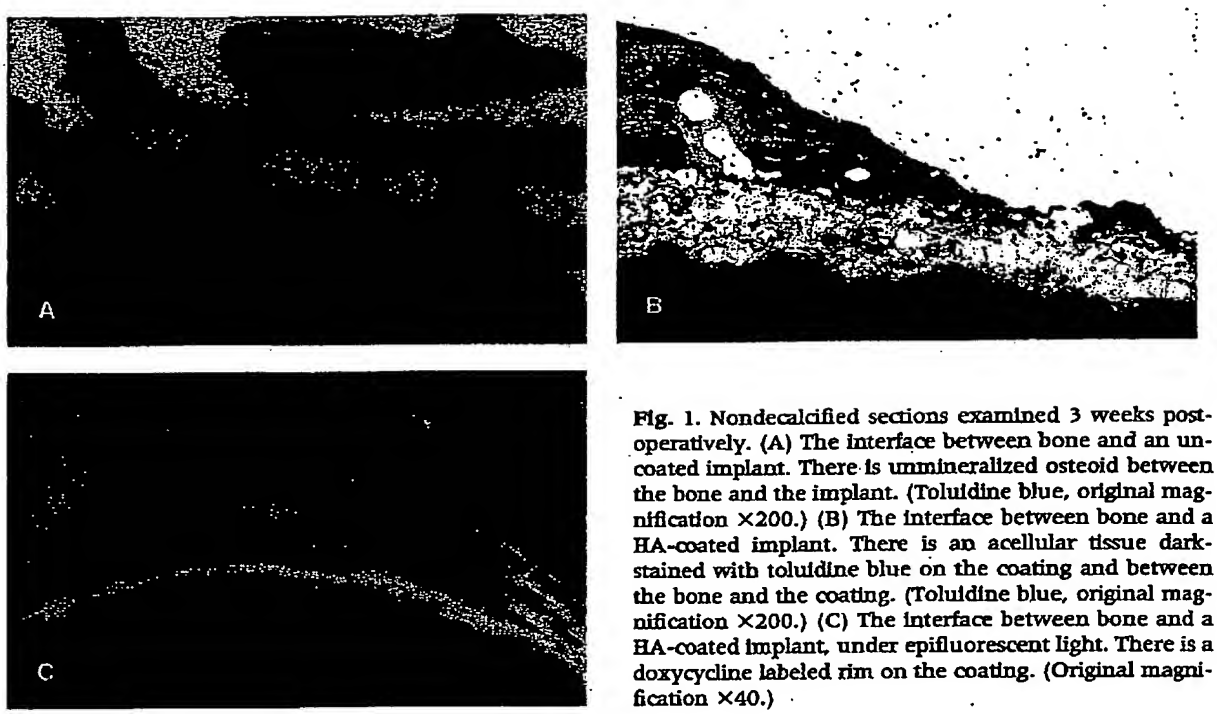

Fig. 1. Nondecalcified sections examined 3 weeks postoperatively. (A) The interface between bone and an uncoated implant. There is unmineralized osteoid between the bone and the implant. (Toluidine blue, original magnification ×200.) (B) The interface between bone and a HA-coated implant. There is an acellular tissue dark-stained with toluidine blue on the coating and between the bone and the coating. (Toluidine blue, original magnification ×200.) (C) The interface between bone and a HA-coated implant, under epifluorescent light. There is a doxycycline labeled rim on the coating. (Original magnification ×40.)

new bone formation between the coated and the uncoated implants. The uncoated implants showed a thin fibrous rim and an unmineralized tissue, such as osteoid or cartilage (Fig. 1A). The unmineralized tissue was seen on at least 1 slide of every femur at 3 weeks and on some slides at 6 weeks but never at 12 weeks. By contrast, the fibrous rim and the unmineralized tissue were never observed on the coated implants. A toluidine-blue stained acellular tissue was seen directly on the coating at 3 weeks with new bone sometimes seen on this acellular tissue (Fig. 1B). This tissue was stained light black by von Kossa staining and labeled by doxycycline (Fig. 1C). The elemental analysis by x-ray energy dispersive spectroscopy detected only calcium and phosphorus in this tissue.

Each implant showed direct new bone attachment at the bone–implant interface by scanning electron microscopy backscatter analysis (Fig. 2). A gap most likely caused by shrinkage artifact was seen between the new bone and the implant surface for the uncoated implants (Fig. 2C), but it was seen between the coating and the substrate metal for the coated implants, suggesting tight bonding at the bone–HA and bone–HA/TCP interface (Fig. 2A, B).

At 24 weeks, the coated implants showed limited areas of focal loss of coating and replacement by new bone, resulting in direct bone contact with the titanium-alloy substrate (Fig. 3). Under epifluorescent light, this new bone was found to be labeled mainly with doxycycline, xylenol orange, and DCAF, administered from the 82nd day to 132nd day postoperatively. The loss of coating was thought to have resulted from resorption rather than delamination because it was not found at earlier periods, and delaminated coatings were never visualized. There was no apparent difference of pattern and amount of resorption between the HA and the HA/TCP coatings during the 24 weeks of observation. The reduction of coating thickness could not be evaluated because of the initial variability of the coating thickness.

Quantitative Histologic Analysis

Percent New Bone in Contact With the Implant. The percentage of the implant perimeter in contact with new bone increased with time (Fig. 4), and the effect of time was significant ($P<.0005$). The section level affected the new bone contact differently between the uncoated and the coated implants (Fig. 5). For the uncoated implants, the new bone contact was significantly ($P<.05$) greater at the diaphysis than at the proximal and distal metaphysis. For the HA-coated and HA/TCP-coated implants, how-

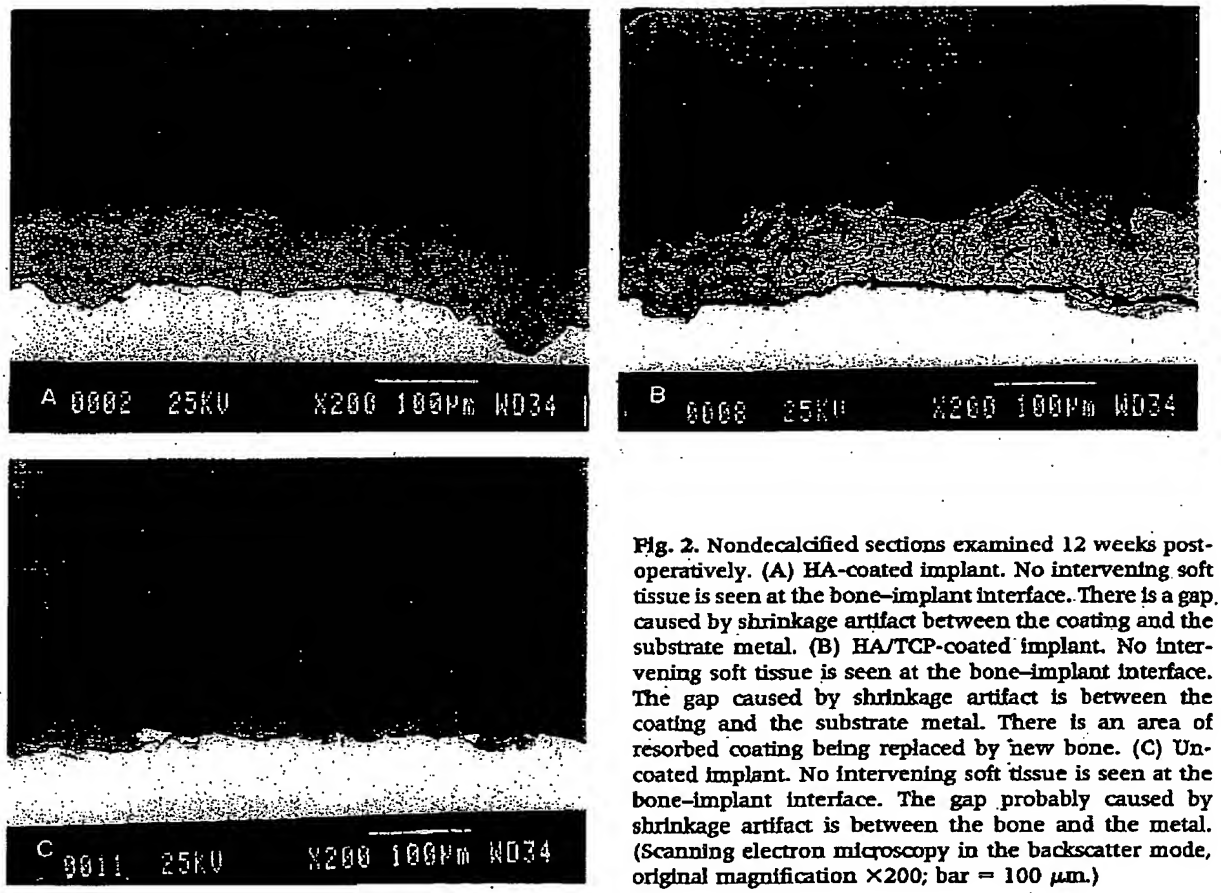

Fig. 2. Nondecalcified sections examined 12 weeks postoperatively. (A) HA-coated implant. No intervening soft tissue is seen at the bone–implant interface. There is a gap caused by shrinkage artifact between the coating and the substrate metal. (B) HA/TCP-coated implant. No intervening soft tissue is seen at the bone–implant interface. The gap caused by shrinkage artifact is between the coating and the substrate metal. There is an area of resorbed coating being replaced by new bone. (C) Uncoated implant. No intervening soft tissue is seen at the bone–implant interface. The gap probably caused by shrinkage artifact is between the bone and the metal. (Scanning electron microscopy in the backscatter mode, original magnification ×200; bar = 100 μm.)

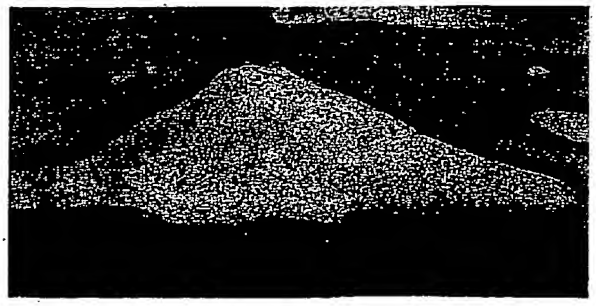

Fig. 3. Nondecalcified section of HA-coated implant examined 24 weeks postoperatively. There is an area of coating resorption and its replacement by new bone, resulting in direct bone contact with the titanium-alloy substrate. (Toluidine blue, original magnification ×200.)

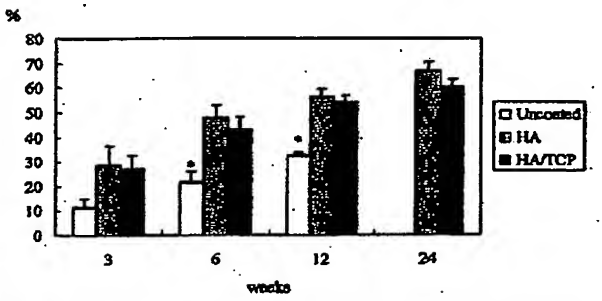

Fig. 4. Percent new bone contact by the time and the coating type. The value increased with time in all of the uncoated, HA-coated, and HA/TCP-coated implants. The data are given as mean ± SEM (n = 6 each) and were analyzed with Student-Newman-Keuls tests and paired *t*-tests with Bonferroni's correction. *Significantly less than the HA-coated and HA/TCP-coated implants at that time period ($P<.05$).

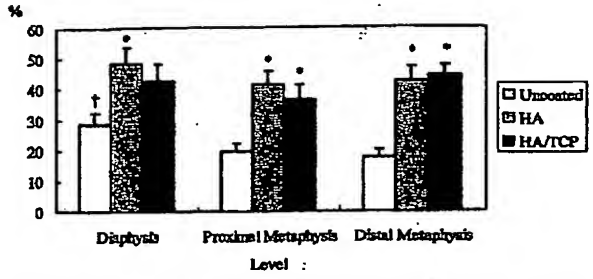

Fig. 5. Percent new bone contact by the section level and the coating type. The data at 24 weeks were excluded. The data are given as mean ± SEM (n = 18 each) and were analyzed with Student-Newman-Keuls tests. *Significantly greater than the uncoated implants at that level ($P<.05$). †Significantly greater than proximal metaphysis and distal metaphysis of the uncoated implants ($P<.05$).

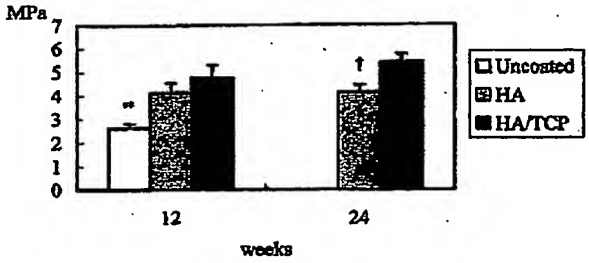

Fig. 7. Graph of the shear strength by the time and the coating type. The data are given as mean ± SEM (n = 6 each) and were analyzed with Student-Newman-Keuls tests and paired *t*-tests with Bonferroni's correction. *Significantly less than HA-coated and HA/TCP-coated implants at 12 weeks ($P<.05$). †Significantly less than HA/TCP-coated implants at 24 weeks ($P<.025$).

ever, the difference of the new bone contact among levels was not significant ($P=.36$). The coating had a significant effect ($P<.0005$); the new bone contact was greater on the coated implants than on the uncoated implants (Figs. 4 and 5). The difference of the new bone contact between the HA-coated and the HA/TCP-coated implants was not significant ($P=.26$).

For the uncoated implants, the unmineralized tissue, such as osteoid and cartilage, was found on 3.8 ± 0.5% of the implant perimeter at 3 weeks, 1.0 ± 0.7% at 6 weeks, and 0.0 ± 0.0% at 12 weeks. Unmineralized tissue was never found on the coated implants at any time.

Percent New Bone Area Around the Implants. The percent new bone area around the implants increased significantly with time ($P<.0005$) (Fig. 6). In contrast to the percent new bone contact, however, the percent new bone area was not affected significantly by the coating ($P=.20$) (Fig. 6). The difference between the HA-coated and the HA/TCP-coated implants also was not significant ($P=.26$). The effect of the section level was significant ($P<.0005$). The percent new bone area was significantly ($P<.0005$) greater at the diaphysis (26.9 ± 2.2%) than at the proximal (11.4 ± 0.9%) and distal metaphysis (12.1 ± 0.8%). There was no significant interactive effect between the section level and the type of coating ($P=.96$).

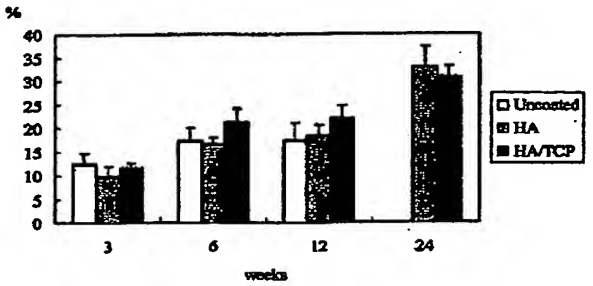

Fig. 6. Percent new bone area around the implant by the time and the coating type. The value increased with time in all of the uncoated, HA-coated, and HA/TCP-coated implants. The data are given as mean ± SEM (n = 6 each).

Mechanical Tests

The bone–implant interfaces of the HA-coated and the HA/TCP-coated implants had significantly ($P<.05$) greater shear strength than that of the uncoated implants at 12 weeks. The HA/TCP-coated implants had greater shear strength than the HA-coated implants, and the difference was significant ($P<.025$) at 24 weeks (Fig. 7). The effect of time on the shear strength of the coated implants was not significant ($P=.45$). Post-test cross-sections of the coated implants revealed that failure occurred at the metal–coating interface, the coating–bone interface, or within bone (Fig. 8). The failure at the metal–coating interface was seen more frequently at 24 weeks (Fig. 8B) than at 12 weeks (Fig. 8A). There was no apparent difference of the failure pattern between the 2 types of coatings. For the uncoated implants, post-test visual inspection of the specimens revealed that failure typically occurred at the implant–bone interface.

Discussion

The HA-coated and the HA/TCP-coated implants showed significantly more new bone attached di-

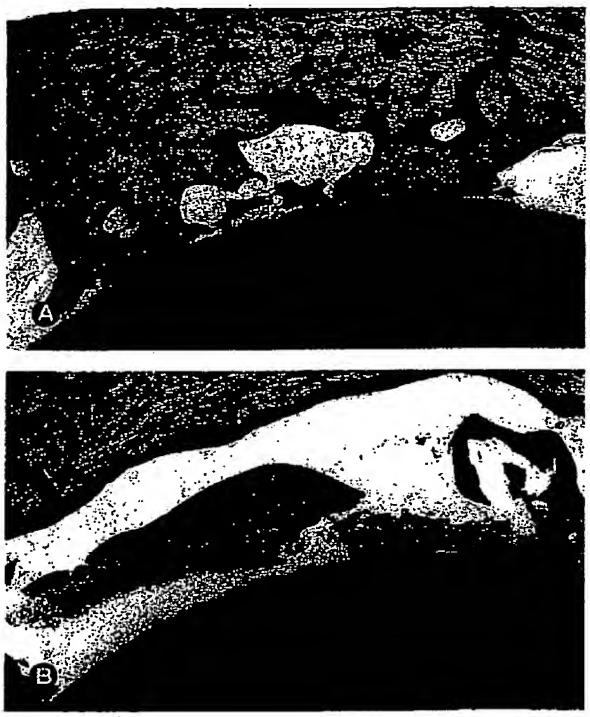

Fig. 8. Nondecalcified sections of HA/TCP-coated implant. (A) 12 weeks postoperatively. Failure has occurred predominantly at the coating–bone interface but also has occurred at the metal–coating interface and within the bone. (B) 24 weeks postoperatively. Failure at the metal–coating interface was seen more frequently. (Toluidine blue, original magnification ×40.)

rectly to the implant surface and a higher interfacial shear strength when compared with uncoated implants. The coatings seemed to eliminate the inhibitory effect against mineralization of the uncoated surface. For both coated implants, bone contact at the metaphyses was comparable with that at the diaphysis and significantly more than that of the uncoated implants.

The difference between the HA and the HA/TCP coatings was the mechanical results. The HA/TCP-coated implants had a significantly higher interfacial shear strength compared with the HA-coated implants at 24 weeks, despite the fact that the percent new bone contact of the HA/TCP-coated implants at 24 weeks was not higher than that of the HA-coated implants. The failure pattern observed after the implant pull-out tests was similar for the 2 types of coated implants. Although this could be accounted for by a difference of bonding strength between the coating and the bone or the implant substrate, it may be the result of the surface roughness differences because the HA/TCP-coated implants had double the measured surface roughness compared with the HA-coated implants. Wong and Eulenberger et al [12] showed an excellent correlation between average roughness of the implant surface and push-out failure load using a transmetaphyseal implant model comparing surface-blasted and HA-coated titanium implants. By contrast, Hayashi and Inadome et al [13] and Inadome and Hayashi et al [14] reported no significant effects of surface roughness on the interfacial shear strength of HA-coated implants. Their implants were transcortical, however, and the values of shear strength were much higher than those reported in this study. Their implants failed by push-out at the coating–substrate interface. It is possible that our results might be different if the implantation site or the observation period were altered.

Another factor that could influence the interfacial strength is the thickness of the coating [1,10,11,15,16]. Yang and Wang et al [16], using a canine model of unloaded, distal femoral intramedullary rods similar to ours, reported higher shear strength for titanium-alloy implants coated with HA of 50-μm thickness compared with those with 200-μm thickness. In our study, the HA/TCP coating was thicker than the HA coating on average, but the failure patterns at the bone–implant interface were similar, and the thicker HA/TCP-coated implants showed even higher shear strength than the thinner HA-coated implants. The slight difference of coating thickness in this study should not have influenced the biomechanical results significantly.

Although it is known that TCP is more soluble than HA, we could not evaluate the direct dissolution of the coatings to extracellular fluid. The areas of focal loss of coatings were seen at 24 weeks with the HA-coated implants and with the HA/TCP-coated implants, but they were seen in only a few areas and could not be evaluated quantitatively. The properties of both coatings used in this study were comparable with those of the commercial coatings currently in clinical use. Our results seem to be consistent with some retrieval studies of clinically stable hip implants [17,18], which suggested cell-mediated HA resorption through bone remodeling.

Although the intramedullary model used in this study is relatively unloaded, the biologic observations provide some clinical relevance. The tissue response to the implants under nonloaded conditions could differ considerably, however, from the response under loaded conditions. Complications of the ceramic coating, such as delamination of the coating and particle production from the coating, might be shown only under weight-bearing condi-

tions. It has been shown that weight bearing accelerates the resorption of the HA coating [19].

The surface texture of the implant substrate metal has a significant effect on interfacial strength. We studied this grit-blasted surface to assess the effect of the HA or the HA/TCP coating on the osseointegration of titanium-alloy implants. We wanted to isolate the effect of the coating because a porous surface might have made it difficult to distinguish the effect of the calcium phosphate coating and the porous surface texture. Additionally, failure of porous surfaces tested in tension may be complex because another interface between the bone and the substrate metal is present. Our pull-out testing results suggested that the coating-substrate interface can be the weakest link between the new bone and the substrate. This suggests a further rationale to use roughened surfaces, which may provide a more robust direct implant fixation to the host.

In this study, the HA coating and the HA/TCP coating enhanced the osseointegration of the grit-blasted titanium-alloy implants. Both coatings had excellent osteoconductivity, and the bone–coating interface showed a tight bond. The weak link that could result in implant fixation failure under clinical weight-bearing conditions is the coating–substrate interface. This study suggests that although further long-term studies in a loaded environment are required, the HA and the HA/TCP coatings are equally effective in enhancing implant fixation.

Acknowledgment

We thank Jay Bensusan, Sarah K. Kirk, and Teresa Pizzuto, for their technical assistance. The support of Sharon Stevenson, DVM, PhD, and Kenichi Shinomiya, MD, throughout this study is especially acknowledged.

References

1. Jaffe WL, Scott DF: Total hip arthroplasty with hydroxyapatite-coated prostheses. J Bone Joint Surg Am 78:1918, 1996
2. Lind M, Overgaard S, Bunger C, et al: Improved bone anchorage of hydroxyapatite coated implants compared with tricalcium-phosphate coated implants in trabecular bone in dogs. Biomaterials 20:803, 1999
3. LeGeros RZ, Orly I, Gregoire M, et al: Substrate surface dissolution and interfacial biological mineralization. p. 77. In Davies JE (ed): The bone-biomaterial interface. University of Toronto Press, Toronto, 1991
4. Kay JF, Cook SD: Biologic profile of calcium-phosphate coatings. p. 89. In Davies JE (ed): The bone-biomaterial interface. University of Toronto Press, Toronto, 1991
5. Bloebaum RD, Beeks D, Dorr LD, et al: Complications with hydroxyapatite particulate separation in total hip arthroplasty. Clin Orthop 298:19, 1994
6. Rokkum M, Brandt M, Bye K, et al: Polyethylene wear, osteolysis and acetabular loosening with an HA-coated hip prosthesis. J Bone Joint Surg Br 81:582, 1999
7. Frayssinet P, Trouillet JL, Rouquet N, et al: Osseointegration of macroporous calcium phosphate ceramics having a different chemical composition. Biomaterials 14:423, 1993
8. Feighan JE, Goldberg VM, Davy D, et al: The influence of surface-blasting on the incorporation of titanium-alloy implants in a rabbit intramedullary model. J Bone Joint Surg Am 77:1380, 1995
9. Jinno T, Goldberg VM, Davy D, et al: Osseointegration of surface-blasted implants made of titanium alloy and cobalt-chromium alloy in a rabbit intramedullary model. J Biomed Mater Res 42:20, 1998
10. Geesink RGT, de Groot K, Klein CPAT: Chemical implant fixation using hydroxyl-apatite coatings: the development of a human total hip prosthesis for chemical fixation to bone using hydroxyl-apatite coatings on titanium substrates. Clin Orthop 225:147, 1987
11. Serekian P: Process application of hydroxylapatite coatings. p. 81. In Geesink RGT, Manley MT (eds): Hydroxylapatite coatings in orthopaedic surgery. Raven Press, New York, 1993
12. Wong M, Eulenberger J, Schenk R, et al: Effect of surface topology on the osseointegration of implant materials in trabecular bone. J Biomed Mater Res 29:1567, 1995
13. Hayashi K, Inadome T, Tsumura H, et al: Effect of surface roughness of hydroxyapatite-coated titanium on the bone-implant interface shear strength. Biomaterials 15:1187, 1994
14. Inadome T, Hayashi K, Nakashima Y, et al: Comparison of bone-implant interface shear strength of hydroxyapatite-coated and alumina-coated metal implants. J Biomed Mater Res 29:19, 1995
15. Wang BC, Lee TM, Chang E, et al: The shear strength and the failure mode of plasma-sprayed hydroxyapatite coating to bone: the effect of coating thickness. J Biomed Mater Res 27:1315, 1993
16. Yang CY, Wang BC, Lee TM, et al: Intramedullary implant of plasma-sprayed hydroxyapatite coating: an interface study. J Biomed Mater Res 36:39, 1997
17. Bauer TW, Geesink RCT, Zimmerman R, et al: Hydroxyapatite-coated femoral stems: histological analysis of components retrieved at autopsy. J Bone Joint Surg Am 73:1439, 1991
18. Tonino A, Oosterbos C, Rahmy A, et al: Hydroxyapatite-coated acetabular components: histological and histomorphometric analysis of six cups retrieved at autopsy between three and seven years after successful implantation. J Bone Joint Surg Am 83:817, 2001
19. Overgaard S, Søballe K, Josephsen K, et al: Role of different loading conditions on resorption of hydroxyapatite coating evaluated by histomorphometric and stereological methods. J Orthop Res 14:888, 1996

Attachment B

Neurosurg Focus 13 (6):Article 2, 2002, Click here to return to Table of Contents

Bone morphogenetic proteins: basic concepts

SETTI S. RENGACHARY, M.D.:

Department of Neurosurgery, Wayne State University School of Medicine, Detroit Medical Center, Detroit, Michigan

The cellular and molecular events governing bone formation in the embryo, healing of a fractured bone, and induced bone fusion follow a similar pattern. Discovery, purification, and recombinant synthesis of bone morphogenetic proteins (BMPs) constitute a major milestone in the understanding of bone physiology. In this review the author discusses the mechanism of action, clinical applications, dosage, and optimum carriers for BMPs. The roles played by other growth factors are also discussed.

KEY WORDS • **bone healing** • **bone morphogenetic protein** • **carrier** • **growth factors** • **spinal fusion**

Bone is unique of all the tissues in the in the vertebrate organism. When injured, it heals by formation of new bone. In contrast, most other tissues such as the heart muscle, voluntary muscles, liver, and the brain heal by replacement of connective tissue rather than the original tissue.

Another interesting attribute of the bone is that the molecular and cellular processes that lead to the development of the skeletal structures within the embryo are very similar to the cascades that occur in the healing process in an injured bone. Likewise, in surgically created fusion, the osseous fusion mass formation recapitulates a fracture healing process, which in turn recapitulates embryonic development of new bone. Thus, there is a common theme in the development of bone from primitive mesenchymal tissues to a well-structured, well-organized histological structure that one associates with mature bone. In addition, the ongoing remodeling process in an adult organism, which is exposed to external physical and hormonal influences, is also modulated through a similar molecular mechanism.

The developing limb bud represents a prototypical example of bone development in the embryo. There is a condensation of primitive mesenchymal cells in the central core of the limb bud, in the area destined to form skeletal structures. This condensation usually transforms into bone through two independent pathways. Intramembranous ossification occurs when there is a direct ossification of the mesenchymal tissues. The primitive mesenchymal cells are transformed into osteoprogenitor cells and then into mature osteoblasts leading to the formation of the bone with all of its histological characteristics.[11] This process occurs typically in the calvarial bones, mandible, and the clavicle. In contrast, the epiphysial growth plate in the appendicular skeleton is characterized by the intracartilaginous bone formation. In this process the primitive mesenchymal cells differentiate in a two-step process into mature bone. In the first step, the mesenchymal cells transform into chondroblasts, form collagen and other elements of bone matrix, become ossified, and lead to mature bone. In the embryonic phase a formation of bone through intramembranous compared with intracartilaginous process depends on the anatomical site. As stated earlier, the cranial structures and mandible undergo an intramembranous growth process whereas the appendicular skeleton forms through an intracartilaginous process.

The postfracture healing of bone generally follows in the intracartilaginous ossification process, although with a very high concentration of BMP an intramembranous route may be taken.[20] At this time, it is unclear what factor(s) direct(s) one process (intramembranous, for example) as opposed to the other in the embryonic phase or during fracture healing. Because the bone that is formed as a result of either process is virtually indistinguishable in its final mature form, it is unclear what the biological advantage is of one process over the other.

Abbreviations used in this paper: BMP = bone morphogenetic protein; ECM = extracellular matrix; FDA = Food and Drug Administration; OP = osteogenic protein; PDGF = platelet-derived growth factor; TCP = tricalcium phosphate; TGF = transforming growth factor.

THE CASCADE OF BONE HEALING FOLLOWING FRACTURE

The stages of bone healing/remodeling are summarized in Table 1. Immediately following a fracture, a hematoma forms at the fracture site due to injury to the periosteum and the adjacent soft tissues. The repair process is initiated with the macrophages are attracted by chemotaxis to the injured site.[3] Macrophages remove the tissue debri whereas fibroblasts generate ECM.[13] A decrease in local oxygen tension and loss of nutrients are factors that promote the release of cytokines and growth factors at the fracture site (Table 2). Degranulating platelets in the hematoma release PDGF and TGFβ. Interleukin-1 and -6 are also released by the inflammatory cells.[2,35] Because of the mitogenic activity of the TGFβ and PDGF, mesenchymal cells and fibroblasts proliferate at the fracture site.[29] The sources of the mesenchymal stem cells are the bone marrow, periosteum, and surrounding soft tissues. Many of these mesenchymal cells are transformed into osteoprogenitor cells by the locally expressed BMPs of various types.[36] Initially chondrogenesis occurs resulting in a soft callus. Calcification of the ECM ensues.[15–19] Angiogenesis is induced concurrently and leads to the formation of woven bone, which then matures into lamellar architecture of the fully developed bone and bone marrow. Remodeling of the bone completes the process.

HISTORY OF BONE MORPHOGENETIC PROTEIN

The history of BMPs is highlighted in Table 3. As early as 1889, Senn[27] noticed that decalcified bone can induce healing of bone defects. He was treating osteomyelitic defects in the bone by using decalcified residue of ox bone with iodoform. His primary goal was use of iodoform as an antiseptic to treat the osteomyelitis, and the decalcified ox bone as was intended a carrier for the iodoform. Serendipitously he noticed that not only was the infection controlled but new bone formed at the osseous defect. Although the observation was interesting, his findings were not easily reproduced by others.

In the 1930s, Levander[9,10] noted that crude alcohol extracts of bone induced new bone formation when injected into muscle tissue. In 1961 Sharrard and Collins[28] reported the use of ethylenediaminetetraacetic acid–decalcified allograft bone for spinal fusion in children. This idea was supported by contemporaneous laboratory studies by Ray and Holloway.[14]

The seminal discovery of the ability of the bone matrix to induce bone was made by Urist in 1965.[30] Urist was director of the bone research laboratory at the University of California, Los Angeles School of Medicine, and was a practicing orthopedic surgeon. He showed that crude bone extracts induced new bone in an ectopic site (in a muscle pouch) in a rat model. He coined the term "bone morphogenetic protein" or "osteogenic protein" which was the active ingredient contained in this extract. His research, however, was hampered by the fact that there was no reproducible assay for the protein. Additionally, it was not conclusively determined that this putative protein was responsible for the induction of new bone in an ectopic site. That task was accomplished by Reddi and Sampath in 1983 when they invented a crude but highly reproducible assay for ectopic bone formation. The assay was based on the activity of alkaline phosphatase and the Ca content of the newly formed bone. This group also showed that when the protein component was dissociated from the matrix,[23] the remaining matrix in itself did not induce new bone formation. When the matrix was reconstituted with the protein, however, it was quite effective as the original matrix in inducing the bone. This conclusively proved that it was not the matrix but actually the protein contained within the matrix that was responsible for ectopic bone formation. The first clinical study was conducted in 1988 by Johnson and associates,[7] who studied purified human BMP. Intensive competition followed in gene sequencing for the BMP. Two groups, one at Creative BioMolecules and the other at Genetics Institute, simultaneously deduced the gene sequence for various BMPs, which resulted in a patent dispute that was subsequently resolved. The human BMP is now produced by using recombinant techniques. Therefore, the available protein is free from the risk of infection or allergic reaction. The cost of the protein, however, remains high. The final landmark in this saga is the FDA approval in 2002 for OP-1 (BMP-7) for long bone defects (Stryker Corp., Kalamazoo, MI) and BMP-2 in a collagen carrier within a cage for anterior lumbar interbody fusions (Medtronic Sofamor Danek, Memphis, TN).

CLASSIFICATION AND CHEMICAL STRUCTURE OF BONE MORPHOGENETIC PROTEIN

Bone morphogenic proteins are members of TGFβ superfamily, a large family of growth factors (Table 4).[4,6,31–34] The TGFβ was so named because of its ability to transform cultured fibroblasts. The BMP subfamily com-

TABLE 1

Stages of bone healing and remodeling

Stage	Characterization
I: induction	formation of hematoma at fracture site: release of growth factors & cytokines
II: inflammation	recruitment of inflammatory cells, macrophages, & fibroblasts to the injury site
III: cartilage formation	mitosis of mesenchymal cells and differentiation of chondrocytes; hypertrophy of chondrocytes & calcification; deposition of extracellular collagenous matrix; local angiogenesis
IV: woven bone formation	differentiation of osteoblasts, mineralization of EM
V: lamellar bone formation	bone resorption, remodeling, formation of lamellar bone & hematopoietic marrow

TABLE 2

Growth factors and cytokines involved in the generation of new bone and remodeling

BMPs
TGF-β
PDGF
insulin-like growth factor SI & II
epidermal growth factor
fibroblast growth factor
vascular endothelial growth factor
Tumer necrosis factor

prises more than 10 proteins, and newer ones are being discovered. There are several structural homologies between BMPs and TGFβ growth factors. The amino acid sequence of BMPs is highly conserved, and is considered to be as old as 600 million years. Because of this conservation, human recombinant BMPs are highly effective in lower life forms, including fruit flies. Like all members of the TGFβ family, BMPs are synthesized as precursor proteins. The precursor protein contains hydrophobic secretive leader sequence as well as substantial propeptides. The mature portion of the protein is located at the carboxy terminal of the precursor molecule. In their carboxy terminal portions, all BMPs contain seven cysteine amino acid residues in positions identical to those present in all members of the TGFβ superfamily. In addition, BMPs contain N-linked glycosylation sites.

The BMP protein can be broadly classified into three subfamilies.[22,24,25] Both BMP-2 and BMP-4 have 80% amino acid sequence homology of molecules. In the second group, consisting of BMP-5, -6 and -7, the mean is 78% amino acid sequence homology whereas the third group, composed solely of BMP-3, is significantly different from the other members of the BMP family and generally stands alone. It is of interest that there is a substantial homology between decapentaplegic peptide and BMP-2 and BMP-4 subfamily, implying that these two BMPs are equivalents of decapentaplegic peptide gene products. Human BMP-6 shows 90% sequence homology across the entire precursor molecule with Vgr-1. There is reason to believe that Vgr-1 is a murine homolog of BMP-6.

The mature segment of the BMPs, which is highly conserved in all organisms, contains seven cysteine amino acid residues. Six of these residues are involved in the formation of intrachain disulphide bonds that forms a rigid "cysteine-knot" molecular structure. The seventh cysteine residue is involved in the formation of dimers via interchain disulphide bond. The dimers may be either homo- or heterodimers. Formation of homo/heterodimers increases the variability of the effector molecule. The reason for this redundancy is not fully understood but probably offers a larger repertoire of molecules with similar functions.

Although BMP is one among the growth factors, it is unique. It is the only morphogen of all known growth factors that has the ability to transform connective tissue cell into osteoprogenitor cells; thus, it is not only a mitogen stimulating the multiplication of connective tissue cells but can be a morphogen, which is able to transform connective tissue cells into osteoprogenitor cells. All other growth factors such as TGFβ, insulin-like growth factor, fibroblast growth factor, PDGF, and vascular endothelial growth factor all induce multiplication of cells but do not transform one cell type into the other.

SIGNALING MECHANISM OF BONE MORPHOGENETIC PROTEINS

The BMP receptors on the cell surface are made up of Type I and Type II serine/threonine kinase proteins. This receptor protein is unique to the TGFβ superfamily of growth factors including BMP. The binding of the ligand to the Types I and II serine/threonine kinase transmembrane receptors results in the formation of heterotetramer complex and activation of the signaling cascade. Immediately after the binding, the Type II receptor kinase phosphorylates the Type I receptor. In turn, the Type I receptor phosphorylates the intracytoplasmic signaling molecules Smads 1, 5, and 8. Following the phosphorylation, Smads 1, 5, and 8 bind to Smad 4 and translocate into the cell nucleus. The entry of the Smads 4/phosphorylated-Smad–1, 5, 8 complex into the cell nucleus results in the activation of transcriptional factors for the early BMP response genes. The BMP signaling cascade, however, is far more complex than indicated by this brief description. There seems to be considerable "cross-talk" between signaling molecules of other growth factors with the BMP signaling complex. This reactivity is undergoing elucidation at this time.

TABLE 3

*Milestones in the discovery and use of BMPs**

Authors/Company & Year	Observation/Discovery
Senn, 1889	decalcified ox bone promotes healing of osteomyelitic defects
Levander, 1934 & 1938	crude alcohol extracts of bone induce bone formation
Sharrard & Collins, 1961	EDTA-decalcified allograft induced spinal fusion in children
Urist, 1965	acid-decalcified bone induced ectopic bone in rat model
Sampath & Reddi, 1981	crude but reproducible quantitative bioassay for BMP; bone matrix when dissociated from BMP ineffective in bone induction; reconstituted matrix effective
Johnson, et al., 1992	purified human BMP successful clinically
Creative BioMolecules & Genetic Institute, 1990s	virtually simultaneous gene sequencing for various BMPs and related patent dispute
Stryker Corp & Medtronic Sofamor Danek, 2002	FDA approval of OP-1 (BMP-7) for long bone defects (Stryker) & BMP-2 in a collagen carrier within a cage for ALIF (Medtronic Sofamor Danek)

* ALIF = anterior lumbar interbody fusion; EDTA = ethylenediaminetetraacetic acid.

TABLE 4
Bone morphogenetic protein family

BMP	Function
BMP-2	osteoinductive, osteoblast differentiation, apotosis
BMP-3 (osteogenin)	most abundant BMP in bone, inhibits osteogenesis
BMPs	
BMP-4	osteoinductive, lung & eye development
BMP-5	chondrogenesis
BMP-6	osteoblast differentiation, chondrogenesis
BMP-7 (OP-1)	osteoinductive, development of kidney & eye
BMP-8 (OP-1)	osteoinductive
BMP-9	nervous system, hepatic reticuloendothelial system, hepatogenesis
BMP-10	cardiac development
BMP-11 (GDF-8, myostatin)	patterning mesodermal & neuronal tissues
BMP-12 (GDF-7)	induces tendon-iliac tissue formation
BMP-13 (GDF-6)	induces tendon & ligament-like tissue formation
BMP-14 (GDF-5)	chondrogenesis, enhances tendon healing & bone formation
BMP-15	modifies follicle-stimulating hormone activity

* GDF = growth/differentiation factor.

Dosage of BMP

It has been estimated that normal bone contains approximately 0.002 mg of BMP per kilogram of pulverized bone. At a fracture site, presumably the BMP is released at a higher concentration because of the secretion by the transformed inflammatory cells into osteoprogenitor cells and upregulation of BMP from the released cytokines at the fracture site. The exact concentration of the BMP at the fracture site as opposed to physiological concentration in the normal bone is unknown.

The concentration required for ideal induced bone bridging in osseous defects depends on several factors. First is the state of the organism in the evolutionary scale. Lower forms such as rodents heal much faster than higher forms such as subhuman primates. Experimental studies involving larger, highly evolved animals require more BMP for the fusion of comparable defects induced in the lower mammals. Additionally, the type of defect should be considered. Simple closed fractures whose ends are apposed by external splinting or internal stabilization do not require any extraneously introduced BMP; is a 99% fusion rate. Apparently, the enhanced BMP released locally at the site of fracture is sufficient to induce bridging of the broken fragments if they are in apposition. Therefore, the use of BMP is seldom considered when treating straightforward long bone fractures. On the other hand, fractures in which there are critical segmental defects in the long bones do not heal spontaneously. They require adjuvant autograft or BMP for adequate healing.

For spine application in the spine, the interbody fusion is less demanding than intratransverse fusion. The latter is well known to be associated with the highest nonfusion rates. The favorable outcome of interbody fusion is related to the fact that drilling of the cortical endplate exposes the bone marrow at the adjacent opposing surface of the fusion, and there is a contiguous supply of bone marrow cells. The gap is seldom more than 13 mm between the vertebral bodies in the lumbar region. The vascularity from the osseous surface is significant. The graft is in the compression mode. All of these factors lead to a favorable outcome with regard to the potential for fusion and require smaller doses of BMP.

In contrast, intertransverse fusion is very demanding. Limited surface area is provided for the exposed cancellous bleeding bone. The linear dimension of the bone to be bridged is generally of the order of 2.5 cm. The bone graft is not loaded. All these factors lead to poor outcome, and in this context the largest doses of BMP need to be used (3–3.5 mg).

The authors of dose escalation studies in animal models have indicated that the superphysiological dose approaching 3 to 3.5 mg of BMP is sufficient in virtually all cases to induce new bone and to bridge the osseous defect. Additional doses of BMP do not confer any benefit in terms of fusion rate or the time taken for the fusion to occur. The quality of BMP-induced bone is comparable with and is indistinguishable from the natural bone. The difference in bone healing when applying BMP is that under normal conditions in the abscence of BMP the bone growth occurs from the bone margins of the gap to be filled and progressively creeps from the ends toward the center; whereas with BMP-induced bone, however, is formed concurrently throughout the defect. Thus, BMP transforms inflammatory cells into osteoprogenitor cells that may freely cross the gap in a concurrent fashion and lay down bone simultaneously to close the gap.

Bone induced under the influence of BMP matures faster than natural healing of the bone. In humans, healing is complete by 8 to 10 weeks as opposed to 12 to 16 weeks when autograft is used.

CARRIERS FOR BMP

Bone morphogenetic protein is a water-soluble relatively low–molecular weight protein that diffuses very easily in the body fluids. When administered in a surgical setting such as in spinal fusion, because the protein will diffuse very rapidly in wound hematomas or can be irrigated away or lost in the suction drainage, it is necessary to contain the BMP. In an experimental setting BMP delivered without a carrier does not endure more than a few hours at the deposited site. It is therefore necessary to contain the BMP in a carrier so that it will have a localized effect at the bone healing site.[26]

The need for a carrier has been recognized since BMP was initially identified. Various carriers have been investigated experimentally and clinically.[8] The BMP carriers can be broadly classified into inorganic salts, naturally occurring polymeric substances, synthetic polymers, and composites of synthetic and naturally occurring polymers.

An ideal carrier should neither induce an inflammatory response nor immune reaction. Degradation of the carrier should not result in toxic residues. Ideally the carrier should be absorbed concurrent with bone healing, leaving no residue. It should be porous, the porosity being equivalent to cancellous bone. The porosity permits trapping of inflammatory cells and bone growth factors. Debate exists regarding the ideal configuration and the sizes of the porosity needed for bone growth. There are competing claims concerning the intercommunicating nature of the porosity with open ends at the surface and the size of the pores. It

is generally agreed that the pore size should be at least comparable with the porosity in the cancellous bone. Pore size even larger than that is thought to be beneficial. Whether the pores should be blind pockets or communicating with each other as well as with the environment is somewhat debatable, and there is no definitive answer to this question.

The most commonly used inorganic salts are Ca phosphate and CaO_4S. The hydroxyapatite, which is a naturally occurring bone mineral and is resorbed rather slowly,[21] is becoming less popular than the alternate TCP. The TCP granules are extensively used as a bone extender. It is absorbed slightly faster (~45–60 days) than the rate of formation of the bone. It can be formulated with varying porosities. Although TCP is a commonly used laboratory chemical, induction of the ideal pore size and geometry requires modification in the synthetic process, which can be a patent-protected intellectual property. Although used experimentally, TCP granules have not been extensively used with BMP in a clinical setting. Phosphate cements are not porous and therefore are not considered as carriers for BMP.

Collagen is the most commonly used carrier, and Type I collagen is preferred. This can be obtained from bone or from tendons and ligaments. Bovine collagen is currently used in the clinical setting as a carrier. It has become apparent from practical use, however, that BMP binds tightly to the bone-derived collagen and not to the tendon-derived collagen. Therefore, the use of BMP-2, which has been approved by FDA for human use, is restricted. Because BMP can be easily squeezed out of the collagen by axial loads and under pressure, it has to be contained in a cage for interbody fusion. Due to fear of the BMP escaping into the epidural space and causing unwanted bone in the epidural location, it has not yet been approved for intertransverse fusion. Osteogenic protein–1 or BMP-7 uses bone-derived collagen, which binds strongly to the BMP presumably through hydrogen bonding. Because of this tighter bonding, OP-1 does not require containment in a cage. Currently OP-1 is used only for long bone defects. It has not been approved for applications in the spine. Demineralized bone matrix as a carrier has not gained popularity because of the risk of immunogenicity and the risk of disease transmission. Other natural polymers that have been considered as a carrier are hyalurone, fibrin, chitoson, alginate, and other animal- or plant-derived polysaccharides. None of these has gained acceptance for human use at the time of this writing.

Synthetic polymers carry the advantage of abundant unlimited supply, low or no antigenicity, predictable absorption, and no risk of disease transmission. Although polyglycolic acid and polylactic acid derivatives have been explored, their degradation products can produce giant cell reaction; the binding affinity of BMP to the synthetic polymers is not as good as that for collagen. Therefore, at present synthetic polymers are not used extensively in humans but are likely to be used when advances in polymer technology occur.

In summary, the most commonly used carrier for human use is Type I collagen. Bone-derived collagen appears to have an advantage over collagen-derived from tendons and ligaments because of tighter binding.

GENE THERAPY

Delivery of the *BMP* gene as a transgene for the target cells remains a potential strategy.[5] The genetic material can be delivered either via a nonreplicating viral vector or nonviral vector. A consistent problem associated with gene therapy has been the low transduction rate. In addition, sustained expression has been difficult to achieve, although with BMP and other growth factors sustained lifetime expression is not needed. Rather, it is needed only throughout the bone healing process. Nonetheless, considerable work is required before gene therapy becomes a reality in a clinical setting.

LONG TERM CONCERNS WITH BMP

There are several long-term concerns about the use of recombinantly manufactured BMPs in humans.[12] The effects of high doses of BMP on a developing embryo are unknown. Therefore, at this time its use during pregnancy is not advised. Although it is a human protein, there is a risk of developing an immune reaction to the protein. This risk increases if BMP is administered more than once such as in cases of repeated fusion. The magnitude of this risk remains unknown.

Development of an osteogenic sarcoma is possible, although experimental dose escalation studies in animal models have not induced neoplasm. It is of some concern that spontaneously evolving osteosarcomas contain high levels of BMPs.

Uncontrollable bone growth in the vicinity of the neural structures, especially nerve roots and cauda equina, is a potential problem. This can only be solved using efficient carriers that bind the protein tightly, preventing BMP release during of protein axial loading.

OTHER USES OF BMP

Bone morphogenetic protein affects organ systems other than bone.[1] It is believed to be a brain protective agent. In fact, in an ischemic rat model, BMP has been shown to offer protection by reducing the size of the infarct. It is an intriguing possibility that in the future BMP may be useful as a protective agent in severe head trauma and stroke.

In patients with chronic renal disease levels of BMP are lower because kidneys are their primary source in the human adult. It is possible that systemic administration of BMP may restore some of the renal functions in patients with chronic renal failure. Additionally, spine surgeons are all too familiar with the renal osteodystrophy syndrome that occurs in patients undergoing long-term dialysis in cases of endstage kidney disease. Fusion rates in these individuals are dismal. Application of BMP locally may be beneficial.

There is considerable ongoing research to determine whether a combination of growth factors (other than BMP) and demineralized bone matrix along with bone marrow aspirate will prove an appropriate substitute for BMP. Recent experimental results are encouraging, but large clinical trials are required to determine if combination therapy will be as efficacious as recombinantly derived human BMP for inducing osseous fusion.

CONCLUSIONS

The cellular and molecular events governing bone formation in the embryo, healing of a fractured bone, and induced bone fusion follow virtually identical patterns. The discovery, purification, and synthesis of BMPs involving recombinant techniques constitutes a major milestone in the understanding of bone physiology. The mechanism of action, clinical application, dosage, and optimum carriers for the protein have been discussed. The roles played by other growth factors have also been discussed.

References

1. Azari K, Doll BA, Sfeir C, et al: Therapeutic potential of bone morphogenetic proteins. **Expert Opin Investig Drugs 10:** 1677–1686, 2001
2. Barnes GL, Kostenuik PJ, Gerstenfeld LC, et al: Growth factor regulation of fracture repair. **J Bone Miner Res 24:**1805–1815, 1999
3. Einhorn TA: The cell and molecular biology of fracture healing. **Clin Orthop 355 (Suppl):**7–21, 1998
4. Elima K: Osteoinductive proteins. **Ann Med 25:**395–402, 1993
5. Helm GA, Alden TD, Sheehan JP, et al: Bone morphogenetic proteins and bone morphogenetic protein gene therapy in neurological surgery: a review. **Neurosurgery 46:**1213–1222, 2000
6. Hoffmann A, Weich HA, Gross G, et al: Perspectives in the biological function, the technical and therapeutic application of bone morphogenetic proteins. **Appl Microbiol Biotechnol 57:** 294–308, 2001
7. Johnson EE, Urist MR, Finerman GA: Resistant nonunions and partial or complete segmental defects of long bones. Treatment with implants of a composite of human bone morphogenetic protein (BMP) and autolyzed, antigen-extracted, allogeneic (AAA) bone. **Clin Orthop 277:**229–237, 1992
8. Kirker-Head CA: Potential applications and delivery strategies for bone morphogenetic proteins. **Adv Drug Deliv Rev 43:** 65–92, 2000
9. Levander G: On the formation of new bone in bone transplantation. **Acta Chir Scand 74:**425–426, 1934
10. Levander G: A study of bone regeneration. **Surg Gynecol Obstet 67:**705–714, 1938
11. McKibbin B: The biology of fracture healing in long bones. **J Bone Joint Surg Br 60:**150–162, 1978
12. Poynton AR, Lane JM: Safety profile for the clinical use of bone morphogenetic proteins in the spine. **Spine 27 (Suppl 1):** 40–48, 2002
13. Probst A, Spiegel HU: Cellular mechanisms of bone repair. **J Invest Surg 10:**77–86, 1997
14. Ray RD, Holloway JA: Bone implants. Preliminary report of an experimental study. **J Bone Joint Surg Am 39:**1119–1128, 1957
15. Reddi AH: Bone and cartilage differentiation. **Curr Opinion Genet Dev 4:**737–744, 1994
16. Reddi AH: Cell biology and biochemistry of endochondral bone development. **Coll Relat Res 1:**209–226, 1981
17. Reddi AH: Role of morphogenetic proteins in skeletal tissue engineering and regeneration. **Nat Biotechnol 16:**247–255, 1998
18. Reddi AH, Ma SS, Cunningham NS: Induction and maintenance of new bone formation by growth and differentiation factors. **Ann Chir Gynaecol 77:**189–192, 1988
19. Reddi AH, Wientroub S, Muthukumaran N: Biologic principles of bone induction. **Orthop Clin N Am 18:**207–212, 1987
20. Ripamonti U, Duneas N: Tissue morphogenesis and regeneration by bone morphogenetic proteins. **Plast Reconstr Surg 101:**227–239, 1998
21. Ripamonti U, Ma S, Reddi AH: The critical role of geometry of porus hydroxyapatite delivery system in induction of bone by osteogenin, a bone morphogenetic protein. **Matrix 12:** 202–212, 1992
22. Sakou T: Bone morphogenetic proteins: from basic studies to clinical approaches. **Bone 22:**591–603, 1998
23. Sampath TK, Reddi AH: Dissociative extraction and reconstitution of bone matrix components involved in local bone differentiation. **Proc Natl Acad Sci USA. 78:**7599–7603, 1981
24. Schaub RG, Wozney J: Novel agents that promote bone regeneration. **Curr Opin Biotechnol 2:**868–871, 1991
25. Schmitt JM, Hwang K, Winn SR, et al: Bone morphogenetic proteins: an update on basic biology and clinical relevance. **J Orthop Res 17:**269–278, 1999
26. Seeherman H, Wozney J, Li R: Bone morphogenetic protein delivery systems. **Spine 27 (Suppl 1):**16–23, 2002
27. Senn N: On the healing of aseptic bone cavities by implantation of antiseptic decalcified bone. **Am J Med Sci 98:**219–243, 1889
28. Sharrard WJ, Collins DH: The fate of human decalcified bone grafts. **Proc Roy Soc Med 54:**1101–1102, 1961
29. Solheim E: Growth factors in bone. **Int Orthop 22:**410–416, 1998
30. Urist MR: Bone: formation by autoinduction. **Science 150:** 893–899, 1965
31. Vukicevic S, Stavljenic A, Pecina M: Discovery and clinical applications of bone morphogenetic proteins. **Eur J Clin Chem Clin Biochem 33:**661–671, 1995
32. Wozney JM: The bone morphogenetic protein family and osteogenesis. **Mol Reprod Dev 32:**160–167, 1992
33. Wozney JM: Bone morphogenetic proteins. **Prog Growth Factor Res 1:**267–280, 1989
34. Wozney JM: The bone morphogenetic protein family: multifunctional cellular regulators in the embryo and adult. **Eur J Oral Sci 106 (Suppl 1):**160–166, 1998
35. Wozney JM, Rosen V, Byrne M, et al: Growth factors influencing bone development. **J Cell Sci Suppl 13:**149–156, 1990
36. Yoo JU, Johnstone B: The role of osteochondral progenitor cells in fracture repair. **Clin Orthop 355 (Suppl):**73–81, 1998

Manuscript received November 29, 2002.
Accepted in final form December 2, 2002.

Address reprint requests to: Setti S. Rengachary, M.D., Department of Neurosurgery, 4201 Saint Antoine, Suite 6E, Detroit, Michigan 48201. email address: srengachary@neurosurgery.wayne.edu.